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LUNG INFLATION AND ALVEOLAR PERMEABILITY TO NON-ELECTROLYTES IN THE ADULT SHEEP *IN VIVO*

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SUMMARY

1. Experiments were performed on adult sheep to determine the effect of lung distension on the passive permeability of alveoli to water soluble non-electrolytes. With the animal breathing oxygen spontaneously, a segment of one lung was isolated by passing a balloon-tipped catheter through a tracheostomy into a distal bronchus. This isolated atelectatic segment was filled with an isosmotic saline solution containing radio-labelled solutes of known molecular size: [^{125}I]albumin, [^{14}C]inulin, [^{14}C]-sucrose, [^{3}H]mannitol, and [^{14}C]urea. The segment was inflated with oxygen either to a preselected pressure, or to a predetermined fraction of its capacity. Inflation was then maintained for several 10–15 min periods between which the oxygen supply was disconnected and the saline sampled, allowing the tracer concentrations to be measured.

2. At low inflating pressures (20–32 cmH_2O) and at low volumes (24–54 % of capacity), alveolar permeability to water soluble solutes was slight and could be characterized in terms of a membrane penetrated by cylindrical water filled pores of 0·5–1·6 nm radius. In all experiments showing restricted diffusion, absorption of saline occurred.

3. There was a positive correlation between the degree of lung inflation and pore radius in both the pressure controlled and volume controlled experiments. At high inflating pressures and at inflation volumes which were close to the total capacity of the isolated segment, restriction of solute diffusion was lost; in five out of six such experiments there was a net movement of liquid into the alveoli.

4. These results can be explained by postulating that as the lung epithelium is progressively stretched there is an opening up of water filled

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channels between alveolar cells. At peak inflation, restriction of diffusion of water soluble solutes is lost, and the alveolar epithelium ceases to function as a barrier between the circulation and air spaces.

INTRODUCTION

In the lung, air is separated from blood by two complete cell layers: the alveolar epithelium and the capillary endothelium. The essential properties required of these structures are that they should be freely permeable to the gases involved in respiratory exchange, O_2, CO_2, and N_2, and at the same time provide a barrier to the net movement of water and solutes from the circulation into the fine air spaces. The first of these properties has been studied extensively, leading to the general conclusion that the permeability of these structures is so great as not to limit gas transfer at sea level. The second property, however, has been little investigated, mainly because of difficulties encountered in measuring solute transfer to and from the very thin film of fluid that exists on the inner surface of alveoli. Because the alveoli of the foetal lamb are filled with a liquid secretion, it has been possible to measure the transfer of test substances put either into the lung liquid or into the circulation across both capillary and alveolar walls and to show that alveolar walls are much less permeable than capillaries to water soluble non-electrolytes (Normand, Olver, Reynolds, Strang & Welch, 1971). In terms of pore theory, the capillary walls of the foetal lamb can be said to be equivalent to a porous membrane containing cylindrical water-filled channels 9·0–15·0 nm in radius, while alveolar walls have characteristics attributable to pores only 0·5–0·6 nm in radius, comparable in solute restriction to other epithelia and intact erythrocyte membranes (Dick, 1970). This degree of solute restriction is not a static property of alveolar epithelium and may be lost under certain circumstances. At the onset of breathing in the lamb, there is a marked increase in alveolar permeability to solutes and water which appears to be related to the increase in lung expansion occurring at that time (Egan, Olver & Strang, 1975). This change in permeability, which can be characterized as an increase in pore radius to a mean of 4·0 nm, may facilitate the absorption of lung liquid at birth. It is, however, a transient change, and by 12–60 h the pore radius falls to 0·9 nm.

Although there is general agreement that alveolar walls are less permeable to solutes than are capillaries, previous work in adult lungs has been confined to isolated perfused preparations which have given widely differing estimates of permeability. Taylor & Gaar (1970) found marked restriction of solute movement and calculated a pore radius of 0·6–1·0 nm for the alveolar epithelium, while other investigators have reported high solute

permeability allowing significant diffusion of macromolecules (Goodale, Goetzman & Visscher, 1970; Wangensteen, Wittmers & Johnson, 1969).

The experiments reported here were undertaken in order to measure the permeability and pore size of the adult lung epithelium *in vivo* and to assess the role of lung inflation as a determinant of the solute permeability of alveolar walls beyond the perinatal period.

METHODS

Experimental procedures

Adult sheep were anaesthetized with I.V. chloralose (3%, 3 ml./kg). A flexible catheter was passed through the right carotid artery into the aorta and a Y-shaped tracheal cannula inserted. The animal was then allowed to breath 100% O_2 for several minutes and a balloon-tipped catheter with a lumen of 2·0 or 2·5 mm diameter was passed through one branch of the tracheal cannula into a distal bronchus. The balloon was inflated with a barium slurry so that its position could be localized by fluoroscopy. With the lumen of its major bronchus occluded, the isolated segment of lung distal to the balloon collapsed as the oxygen within it was absorbed while the animal continued to ventilate spontaneously the remaining area of the lung. Following complete collapse of the isolated segment, 20–150 ml. saline solution, isosmotic with the animal's plasma and containing radiolabelled tracers, were introduced through the balloon catheter. The volume used was empirically determined by the size of the segment shown to be isolated on fluoroscopy and the necessity of being able to withdraw easily a sufficient volume for sampling. After a 3 min period to allow for complete osmotic equilibrium, the saline was withdrawn and a 0·5 ml. sample taken together with 2 ml. blood from the carotid artery. Following reintroduction of the saline solution the isolated segment of the lung was inflated with O_2, either to a preselected pressure or predetermined volume. In either case, inflation was controlled by displacement of oxygen from an aspiration bottle by water run in from a graduated burette.

Inflation of the isolated segment was maintained for 10 or 15 min by a constant positive airway pressure of O_2. During the inflation period, there was some cyclic variation in the transpulmonary pressure in the isolated segment due to spontaneous ventilation of the animal and a small net influx of gas due to O_2 absorption. At the end of the inflation period, the lumen of the catheter was closed, some of the O_2 aspirated, and the rest was absorbed by the blood. The saline was withdrawn, mixed, sampled and reinfused, and the isolated lung segment reinflated as before. Three to five such inflation periods were accomplished in each experiment. In all animals, blood samples were taken from the carotid artery at the same time as the lung saline samples for isotope counting and periodic measurement of pH, p_{CO_2}, and p_{O_2}. Blood pressure, heart rate, and airway pressure in the isolated segment were monitored continuously.

Control of inflation

In the first set of experiments, lung inflation was controlled by exposing the isolated segment for 2 min to a specific inflation pressure between 20 and 45 cmH_2O. These pressures are comparable to those needed statically to inflate foetal lungs which are filled with lung liquid (Egan *et al.* 1975). After 2 min at the selected inflation pressure, the isolated segment was attached to a constant O_2 flow under 18 cmH_2O pressure. Thus, all the animals were studied at the same pressure on the

descending limb of an inflation loop, but the gas volume in the isolated segment at that pressure was dependent on the initial inflation pressure.

In the second set of experiments, total volume (saline plus gas) was controlled. After the saline and tracers had been introduced into the isolated segment, the burette was raised 30–40 cm above the water level in the aspirating bottle, and, over a period of 20–40 sec, lowered by hand until the desired gas volume had been reached. The pressure–volume relationship during this phase was, therefore, dynamic and not comparable with the static peak inflations used in the pressure controlled experiments. It was found that a positive pressure of 10 cmH_2O was adequate to maintain the gas volumes in these experiments with a minimum of gas leakage. Any loss of O_2 by leakage or consumption was made good by continuously raising the burette to maintain the inflation pressure at 10 cmH_2O. In general, the amount added in this way during the inflation period did not exceed 20% of the initial gas volume.

The total volume (saline plus gas) contained within the isolated segment was expressed as % total capacity. However, direct measurement of the total capacity of the segment was not possible because it would have necessitated an initial inflation to maximum volume in every case, and because at high inflation pressures some leakage of gas around the balloon did occur. To estimate the volume of the airspace in the isolated segment, a minimum gas volume of the segment was determined before infusion with the saline. This volume was measured by inflating the atelectatic segment with a known volume of O_2 at a pressure of less than 25 cmH_2O and then withdrawing as much as possible with a small negative pressure into a syringe. After an inflation, several minutes were allowed for the residual O_2 in the isolated segment to be absorbed into the blood, and another inflation with a different volume was performed. The minimum gas volume was determined as the volume of O_2 which could not be withdrawn and which did not vary more than 10% with different inflation volumes.

We then assumed this minimum gas volume to be 25% of the total capacity of the airspace on the basis of the relationship shown to exist between residual volume and total lung capacity in man and dogs (Milic-Emili, 1974) and direct measurements made *post mortem* in two of our sheep on atelectatic lungs which gave values for minimum gas volume of 23 and 26% of total capacity.

Radioactive test substances

The following radiolabelled tracers were added to the saline solution introduced into the isolated segment of lung: [^{125}I]albumin (a_s = 3·4 nm), [^{14}C]inulin (a_s = 1·39 nm), [^{14}C]sucrose (a_s = 0·51 nm), [^{3}H]mannitol (a_s = 0·42 nm), and [^{14}C]urea (a_s = 0·22 nm). The corrected molecular diffusion radii, a_s, are taken from Normand *et al.* (1971) and Boyd, Hill, Humphreys, Normand, Reynolds & Strang (1969).

Stock solutions of the ^{14}C and ^{3}H-tracers were made up with 2 m-mole/μc of unlabelled substrate as carrier. The [^{125}I]albumin had 10 mg/μc unlabelled substrate as carrier. To 100 ml. saline solution 3–15 μc of each tracer were added, relatively more being used for the smaller molecules with higher diffusion coefficients and for mannitol which, with its tritium label, is counted less efficiently.

Analytical procedures

Lung saline and plasma samples were centrifuged for 20 min at 20,000 *g*. A 0·1 ml. aliquot of each was counted on a Packard Autogamma Tricarb Scintillation Spectrometer at 28 keV to measure ^{125}I activity. At least 10^4 counts above background were obtained on each lung saline sample. The plasma samples, which had very low ^{125}I activity, or none at all, were counted for 10^4 sec. ^{125}I counts on consecutive samples were corrected to a standard time.

The β emitting test substances, all of which were ^{14}C-labelled apart from mannitol, were separated by gel filtration on sephadex G-15 and G-50 columns. The inulin peak of the G-50 eluate and the sucrose and urea peaks on the G-15 eluate were distinct, and no other ^{14}C labelled substance contributed to the height of these peaks. In both columns, [^{125}I]albumin appeared at the void volume. Inulin appeared in the void volume of the G-15 column, but in a gaussian peak on G-50. The other tracers, sucrose, mannitol, and urea, appeared in distinct gaussian distributions on G-15. The size of the peak was determined by averaging the counts in the three 0·3 ml. fractions with the highest activity for each trace molecule. The height of the peak of single labelled test substances individually applied to either column was directly proportional to the total amount of the labelled tracer ($\pm 2\%$).

Separate 0·1 ml. aliquots of each lung liquid sample were applied to both the G-50 and the G-15 columns. Each 0·3 ml. fraction of the eluate was mixed with 15 ml. PCS solubilizer (Amersham Searle) and its activity was determined by counting on a Packard Tricarb Liquid Scintillation Spectrometer. In fractions with both [^{3}H]mannitol and [^{14}C]sucrose, the individual counts were obtained by a channel separation procedure employing the solution of two simultaneous equations with coefficients derived from ^{3}H and ^{14}C standards counted with the experimental samples. In addition, a 0·5 ml. aliquot of each plasma sample was mixed with 15 ml. PCS solubilizer. Only [^{14}C]urea counts were detected in plasma at more than 1 % of lung saline counts, and then only in late samples. When present, they were subtracted from [^{14}C]urea counts in the lung saline sample to give the concentration difference across the epithelium.

RESULTS

Restricted diffusion

Irrespective of how the initial period of inflation was controlled, by pressure or volume, in most of the experiments the pattern of change in concentration of tracers with time appeared as illustrated in Fig. 1. In Fig. 1, which is taken from an experiment performed with an inflation pressure of 34 cmH_2O, the concentrations of both albumin and inulin rose while those of the smaller solutes fell at rates inversely proportional to their molecular radii. The albumin and inulin slopes are not significantly different, in spite of the fact that their free diffusion coefficients vary by a factor of 2·5 and we may conclude that both substances are completely restricted by the epithelium; furthermore, neither tracer was detectable in plasma. The upward slope of albumin and inulin (γ) can be interpreted as being due only to absorption of saline into the circulation while the slopes of sucrose, mannitol, and urea must reflect the algebraic sum of absorption of liquid and diffusion of tracer out of the lung saline across alveolar walls ($K_0+\gamma$). At no time did even the smallest tracers in plasma exceed 5 % of their concentration in lung saline and, since capillary walls are much more permeable to solutes than alveoli, we can write the following expression to describe the change in concentration with time of the permeant tracers in lung saline (see Olver & Strang, 1974):

$$\ln C_L = \ln C_{L,0} - (K_0+\gamma)\, t, \qquad (1)$$

where $C_{L,0}$ is concentration, C, at 0 time; K_0 is a rate constant (min^{-1}) for passive one way transfer from lung saline to plasma; $\gamma = J_v/V$, where J_v is the rate of volume change of alveolar liquid in ml. min^{-1} and V the mean volume in ml. liquid in the isolated segment during the period of measurement. A negative value of γ is obtained from the positive slope of an impermeant tracer (here albumin and inulin) and indicates liquid absorption. Since the amount of impermeant tracer added to lung saline is known, the alveolar saline volume at any point in time can be readily calculated.

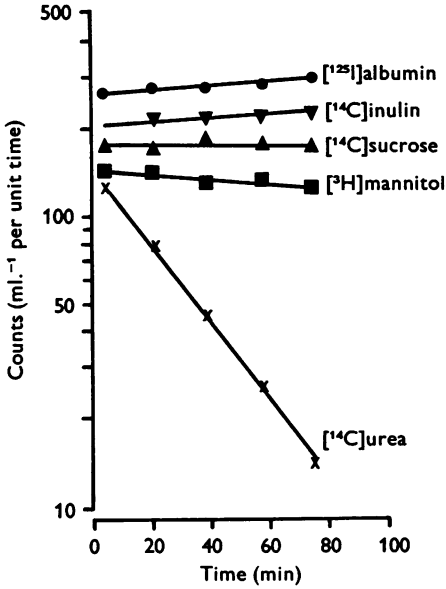

Fig. 1. Expt. 1070. Abscissa: time from infusion of saline and tracers into the airspace. Ordinate: counts in lung liquid ml.$^{-1}$ per unit time. The regression lines fitted by best least-squares method.

Calculation of pore radius

For any given degree of solute restriction, the K_0 for a test substance will vary with both the area for diffusion and the volume of the isolated segment ($K_0 :: A/V$). Although volume can be calculated, area is unknown, and thus comparisons between one experiment and another cannot be made on the basis of K_0 values alone, nor can the lung epithelium be compared to other epithelia on this basis. However, this difficulty can be overcome if the permeability of epithelial walls is expressed in terms of a membrane containing uniform water-filled cylindrical pores. According to pore theory, where transfer is determined by restricted diffusion (Normand *et al.* 1971):

$$K_0 = D\,[(A/\Delta x)/V]\,F(a/r), \qquad (2)$$

where D is the free diffusion coefficient at experimental temperature, $A/\Delta x$ is pore area per unit path length, V is volume of saline in the air-spaces, and $F(a/r)$ is the Faxen–Ferry function relating molecular radius, a, to pore radius, r (Solomon, 1968). Values for $F(a/r)$ vary from 0 for impermeant tracers to 1 for free diffusion. Fig. 2 shows the K_0 values from Fig. 1 plotted against molecular radius and three lines calculated from eqn. (2) for different pore radii. The data are best fitted by the line calculated for pores of 0·9 nm radius.

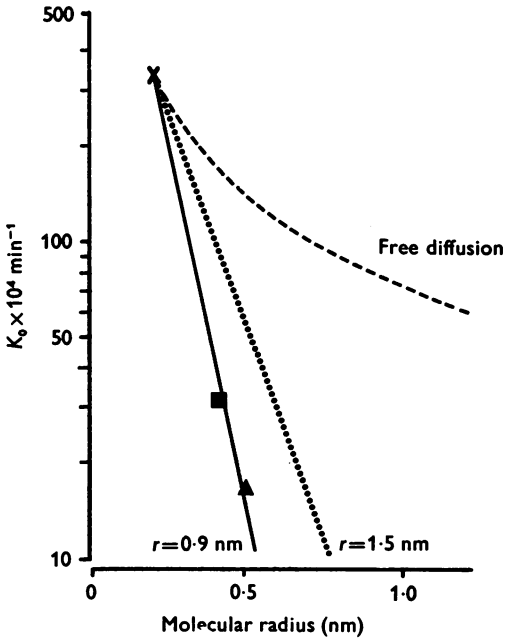

Fig. 2. Relationship betwen transfer constants K_0 and molecular radius obtained in expt. 1070 (Fig. 1). Abscissa: molecular radius in nm. Ordinate: K_0 in min^{-1}. Continuous line shows the prediction of eqn. (2) for diffusion limited to pores of 0·9 nm radius, dotted line for pores 1·5 nm radius, dashed line is that expected for free diffusion unrestricted to a pore radius. All three lines were made to pass through the experimental urea point which was taken to define $(A/\Delta x)/V$. The points are the experimental K_0 values for urea (×), mannitol (■), and sucrose (▲).

Free diffusion

In other experiments the concentration of all tracers fell, as in Fig. 3 which shows the results obtained after inflating the isolated segment to total capacity. The fact that the ratios of the slopes of the test substances are less than the ratios of their free diffusion coefficients must imply that there has been an expansion of the volume of liquid within the isolated

segment during the experimental period. However, the fall in concentration of albumin and inulin cannot be due solely to dilution by bulk flow of liquid from the circulation since their slopes are different, and low counts of [^{125}I]albumin were detected in the blood. Loss of tracer by diffusion must have occurred. Since all tracers are diffusing out, a direct calculation of γ cannot be made, but an estimate can be obtained if we first make the assumption that the rates of transfer of albumin and urea are governed

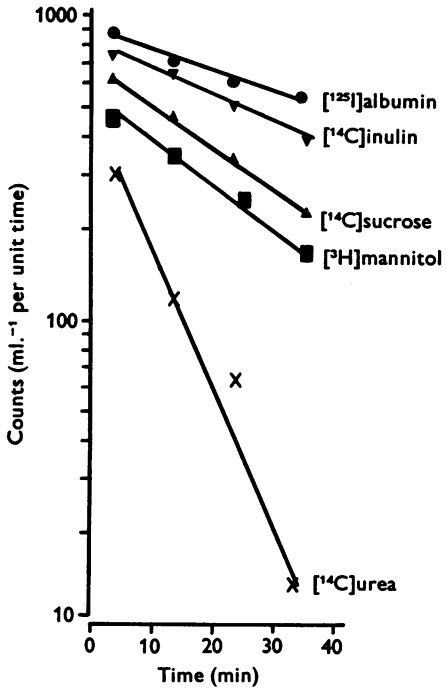

Fig. 3

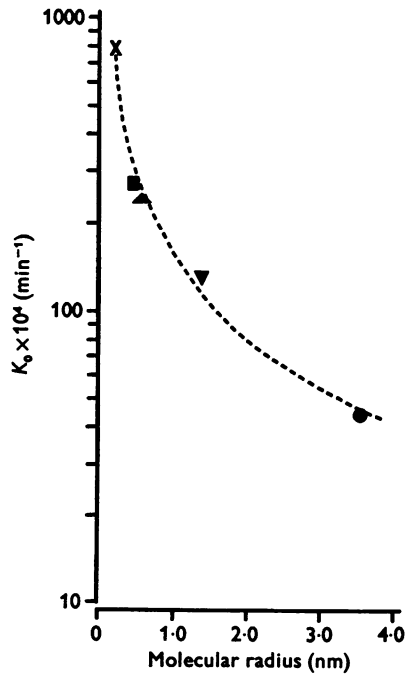

Fig. 4

Fig. 3. Expt. 2041. Abscissa: time from infusion of saline into isolated lung segment. Ordinate: counts in lung liquid ml.$^{-1}$ per unit time. The regression lines fitted by least-squares method.

Fig. 4. The relationship between transfer constants, K_0, and molecular radius in expt. 2041. Abscissa: molecular radius in nm, Ordinate: K_0 in min^{-1}. The line is the predicted relationship for free diffusion and was made to pass the urea point which was taken to define $(A/\Delta x)/V$. The points are the experimental K_0 values for albumin (●), inulin (▼), sucrose (▲), mannitol (■), and urea (×).

only by their free diffusion coefficients and that for the smallest molecule K_0 is much larger than γ so that for urea an approximation is made: $K_0 \approx K_0 + \gamma$. Since $(A/\Delta x)/V$ is a constant for all tracers in any one experi-

ment and $F(a/r) = 1$ for unrestricted diffusion (see eqn. (2)), we can estimate K_0 albumin from the expression:

$$[K_0 \text{ urea}]/[D_s \text{ urea}] = [K_0 \text{ albumin}][D_s \text{ albumin}]. \quad (3)$$

The value of K_0 obtained in this way can then be used to extract γ from the experimental albumin slope, and, in turn, K_0 values for the other solutes can be determined. In Fig. 4, the tracer K_0 values from Fig. 3 are plotted against molecular radius and compared with the predicted relationship between K_0 and molecular radius for free diffusion. Clearly there is no restriction to solute movement imposed by the alveolar epithelium. Although the method of calculating γ in this experiment means that both the albumin and the urea points will approximate the predictions for free diffusion, there is no such contraint on the other tracers.

Inflation pressure and alveolar pore radius

In the fourteen experiments in which the saline-containing segment was first inflated to a preselected pressure (20–40 cmH_2O) before being held at a constant airway pressure of 18 cmH_2O, pore radius tended to increase with increasing pressure (Table 1). In three experiments, free diffusion of solutes was observed, and in two of these the net volume flow was from the circulation into lung saline (i.e. γ positive). In all other experiments in this group γ was negative – saline was absorbed from the isolated segment. Although there was a significant positive correlation between inflating pressure and pore radius (Fig. 5), considerable variability was observed between different animals inflated to the same pressure, implying the existence of substantial differences in lung distensibility.

Inflation volume and alveolar pore radius

The variation in results in the pressure-limited inflation experiments prompted a second series of observations in which the volumes of liquid and gas in the isolated segment were controlled (see Methods). The results of these experiments are shown in Table 2. The positive correlation between inflation volume (% total capacity) and pore radius seen in Fig. 6 ($r = 0{\cdot}819$, $P < 0{\cdot}005$) is much better than in the pressure-limited experiments ($r = 0{\cdot}624$, $0{\cdot}01 < P < 0{\cdot}05$). Pore radii greater than 1·5 nm were observed only at lung segment volumes more than 60 % of total capacity, and only at 100 % of capacity was unrestricted diffusion seen. Saline was absorbed from the isolated segment except in the three experiments in which restriction of diffusion was lost when γ reversed and became positive.

In both the pressure and volume controlled experiments in which pore radii were calculated the data in each case were compatible with the existence of pores of uniform dimensions or, more likely, a population of

TABLE 1. Results of pressure controlled experiments

Expt. no.	Inflation pressure (cmH_2O)	Initial lung liquid vol. (ml.)	γ ($\times 10^4$)	K_0 ($\times 10^4$)					Pore radius (nm)
				Albumin	Inulin	Sucrose	Mannitol	Urea	
1020	20	50	−2·2	0	0	−2·3	−17·5	−408·2	0·6
1141	20	102	−4·0	0	0	−12·5	−49·2	−266·2	0·9
1030	23	90	−15·3	0	0	−8·6	−30·6	−476·8	0·8
1142	30	94	−47·3	0	0	−61·5	−116·5	−293·9	1·6
1110	32	100	−14·9	0	0	−43·2	−58·4	−495·5	1·1
1060	34	100	−30·2	0	−14·3	−166·6	−201·4	−474·4	3·5
1070	34	100	−13·2	0	0	−17·2	−31·9	−323·2	0·9
1130	34	100	−10·7	−33·5	−69·5	−197·5	−270·5	−609·1	leak
1172	35	76	−10·0	0	−4·6	−48·6	−57·8	−391·1	2·7
1090	36	73	+5·0	−35·5	−126·0	−239·8	−284·8	−589·4	leak
1171	38	74	−14·2	0	−3·7	−81·8	−97·3	−338·1	2·5
1160	38	150	+37·9	−25·2	−83·0	−205·9	−239·2	−447·4	leak
1150	40	106	−27·2	0	−42·5	−94·4	−130·4	−375·2	5·0
1140	43	31	−18·2	0	0	−32·2	−60·6	−381·2	1·6

γ = Net flux of lung liquid per unit volume (min^{-1}).
K_0 = Transfer constant (min^{-1}).

TABLE 2. Results of volume controlled experiments

Expt. no.	Inflation vol. % capacity	Initial lung liquid vol. (ml.)	γ ($\times 10^4$)	K_0 ($\times 10^4$)					Pore radius (nm)
				Albumin	Inulin	Sucrose	Mannitol	Urea	
1222	25	52	−0·8	0	0	−27·7	−42·6	−411·7	0·9
1252	32	150	−33·8	0	0	−28·9	−40·6	−508·4	0·9
1221	35	25	−43·7	0	0	−16·3	−68·7	−423·8	0·9
1240	50	50	−6·0	0	0	−5·1	−19·4	−577·4	0·6
1251	50	51	−20·5	0	0	−42·6	−92·9	−710·4	1·0
1282	50	21	−4·7	0	0	−18·5	−50·9	−253·5	1·1
1281	54	75	−21·8	0	0	0	−24·7	−344·4	0·5
2022	62	30	−153·2	0	−90·3	−237·7	−310·2	−929·5	2·1
1191	63	35	−70·5	0	0	−75·4	−89·7	−219·5	0·9
1273	75	14	−41·4	0	−36·8	−144·1	−167·5	−582·1	3·0
1261	87	100	−13·2	0	−34·6	−45·1	−71·7	−450·8	2·0
1262	90	100	−17·5	0	−13·5	−136·0	−183·6	−572·2	3·5
1192	92	29	−20·3	0	−45·5	−162·8	−285·9	−781·5	4·0
2041	100	49	+93·8	−76·1	−131·1	−245·6	−275·8	−745·0	leak
2021	100	20	+178·9	−44·2	−120·6	−261·8	−308·9	−724·1	leak
2030	100	20	+168·9	−33·0	−99·8	−291·5	—	−552·9	leak

γ = Net flux of lung liquid per unit volume (min^{-1}).
K_0 = Transfer constant (min^{-1}).

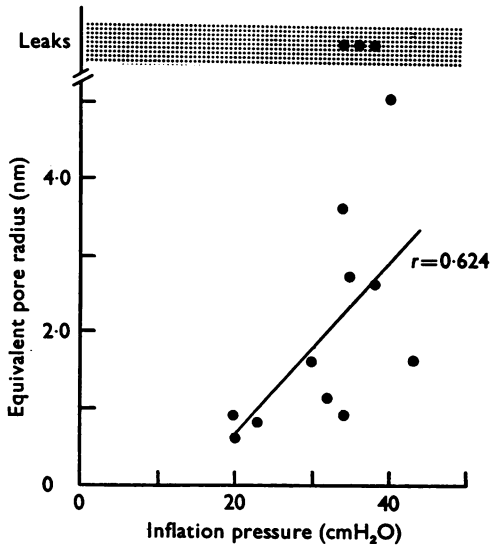

Fig. 5. Pressure-controlled inflation. Ordinate: equivalent pore radius of alveolar epithelium (nm). Abscissa: static inflating pressure. Regression line fitted by least-squares method. Experiments showing leaks not included in regression calculation.

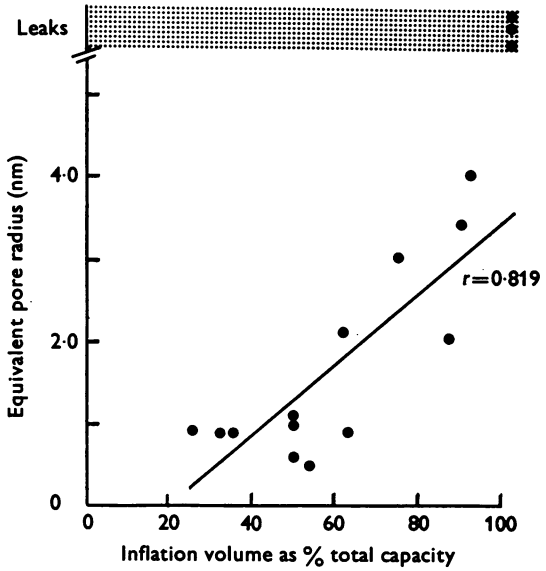

Fig. 6. Volume-controlled inflation. Ordinate: equivalent pore radius of the alveolar epithelium (nm). Abscissa: total volume of inflation, liquid and gas, expressed as a percentage of the total capacity of the airspace of the isolated lung segment. Regression line fitted by the least-squares method. Experiments showing leaks not included in regression calculation.

pores closely scattered around a mean value. In general the data points fit closely the predictions for a single pore radius as in Fig. 2 and there is no evidence to suggest the presence of two or more populations of pore radii such as might occur as a result of gross inhomogeneity of lung expansion or overall change in volume during each inflation period.

DISCUSSION

From the data presented here it can be seen that the alveolar permeability of the adult lung is not a static property but varies with the degree of lung inflation. At moderate lung volumes the permeability of solutes is slight; the measured pore radius of aveolar walls is similar to that described in newborn lambs several hours after birth (Egan *et al.* 1975) and is not much greater than that observed in foetal lambs (Normand *et al.* 1971). However, at high lung inflations, pore radius increases so that the degree of solute restriction falls to levels described in the newborn lungs at the onset of ventilation (Egan *et al.* 1975). At total lung capacity all restriction of solute transfer is lost and free diffusion occurs.

Thus, under normal conditions, alveolar walls exhibit a property possessed by epithelia in general: that of selective permeability and, by analogy with other epithelia, the most important site of solute and water penetration may well be the intercellular junctions (Frömter & Diamond, 1972). Our assertion that the adult alveolar epithelium severely restricts the movement of small solutes (including ions) is compatible with studies using the electron microscope which show that horseradish peroxidase cannot pass between adjacent alveolar cells (Schneeberger-Keely & Karnovsky, 1968) and that on freeze-fracture the intercellular junctions appear complex and 'tight' (Schneeberger, 1976). The observation that at high lung volumes solute restriction declines suggests that at high inflations the clefts between alveolar cells are pulled apart and, although at first the spaces retain molecular dimensions, there is eventually complete separation of cells at maximum lung capacity. Reversible changes such as these have been shown by electron microscopy to occur in the frog cornea exposed to a hydrostatic pressure of 18 cmH_2O (Zadunaisky, 1971).

The high degree of solute restriction shown by the intact lung epithelium has both theoretical and practical importance. As discussed by Egan *et al.* (1975), a large osmotic pressure difference may be generated across alveolar walls which restrict Na^+ and Cl^- movement when hyper- and hypotonic solutions enter the lung as in drowning. When isotonic liquid is introduced into the air spaces, as in our experiments, absorption of liquid takes place because of the difference in protein osmotic pressure generated across an epithelium which completely restricts the diffusion of proteins,

i.e. it acts as a semipermeable membrane. However, when this property is lost, as it was in those experiments in which the lung was hyperinflated and leaks were produced, the gradient of osmotic pressure is lost and, in five out of six such cases, fluid passed from the circulation into the saline filled airspaces. It appears that free solute diffusion represents injury of the lung epithelium, accompanied by loss of its capability to restrict water and solutes to the interstitium, and that maintenance of restricted diffusion to solutes is an essential property of the epithelial lining.

Recently, it has been claimed (Staub, 1976) that the terminal airway epithelium is normally quite permeable to proteins. However, this conclusion is based on experiments in which isolated lung lobes were filled with isosmotic fluid containing tracer albumin and 10 and 20 cmH_2O airway pressure. Unlike our experiments, there was no air-liquid interface interposed between the pressure source and the lung epithelium, and, therefore, in the absence of surface tension, we can expect the whole of this considerable static force to have been exerted on alveolar walls. In foetal lambs, Egan *et al.* (1975) noted that saline distension of the lung at a pressure of 15 cmH_2O (no air-liquid interface) disrupted the normally tight alveolar epithelium, giving rise to unrestricted diffusion. Pressures of this order have been shown to produce marked increases in permeability in various epithelia and are associated with important changes in function. Absorption of fluid from the dog intestine ceases when pressures as low as 4 cmH_2O are applied to its serosal surface (Hakim & Lifson, 1969). At 10 cmH_2O, the direction of net flow is reversed, and first inulin, then Evans blue, ferritin, and finally erythrocytes enter the mucosal fluid. Similarly, the *Necturus* renal tubule exposed to peritubular pressures up to 6 cmH_2O during saline loading shows a large increase in the paracellular shunting of solutes which results in an increased Na^+ backflux into the tubule lumen and consequent natriuresis (Grandchamp & Boulpaep, 1974). Changes analogous to these may be responsible for alveolar flooding in pulmonary oedema when interstitial pressure reaches a critical level. However, we cannot predict the magnitude of this critical pressure from the results of lung inflation experiments since the change in permeability of an epithelium in response to increasing pressure may not be symmetrical (Hakim & Lifson, 1969). Furthermore, the presence of an air-liquid interface in our experiments makes such an extrapolation impossible.

There clearly must be an increase in lung epithelial permeability in pulmonary oedema, since all analyses of pulmonary oedema fluid show it to contain large amounts of protein (Staub, 1974). Other situations in which the barrier function is lost are the adult and infant respiratory distress syndromes in which hyaline membranes, containing large proteins are seen (Gitlin & Craig, 1956). Continuous transpulmonary pressure ventila-

tion techniques have proved effective in improving the hypoxaemia in these conditions (Suter, Fairley & Isenberg, 1975; Gregory, Kitterman, Phibbs, Tooley & Hamilton, 1971). and in the treatment of hypoxaemia secondary to respiratory failure positive expiratory pressures as high as 44 cmH_2O have been used (Kirby, Downs, Civetta, Modell, Dannemiller & Klein, 1975). Our experiments suggest that hyperinflation induced by such techniques could increase the passive permeability of some areas of the lung and, as a result, alter the balance between osmotic and hydrostatic forces acting across the alveolar epithelium. In the extreme case, hyperinflation may cause disruption of alveolar walls, with the resultant free diffusion of solutes and water complicating the original lung pathology.

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